

## EFFECT OF A FOOD ADDITIVE ON CERTAIN HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN MALE ALBINO RAT

MAZEN ATTIA ALSOLAMI

Department of Biology, College of Haqel, University of Tabuk, KSA

### ABSTRACT

*Food additives are used for various purposes, including preservations, colouring and sweetening. Some food additives, however, have been prohibited from use because of their toxicity. Azo dyes are one of these food additives which widely used as colorants in foods. The present study was conducted to evaluate the possible influence impacts of an azo dye (allura red) on some physiological and biochemical parameters of male albino rat *Rattus norvegicus*. So, forty adult male rats weighing 100-110 g, were divided into 4 groups, the first and third groups were served as controls, the second group received 50 mg/kg, bw. of allura red for 10 days and the fourth group was treated with 50 mg/kg, b.w. of allura red for 40 days. All rats groups were treated orally. The data obtained reveal a marked decrease in red blood cells (R.B.Cs) counts, haemoglobin (Hb) content; mean corpuscular hemoglobin concentrations (MCHC) of rats treated with allura red. On the other hand, a noticeable increase in haematocrit (Hct) value, mean corpuscular volume (MCV), activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose level, serum total protein and globulin were found in rats treated with allura red.*

*In conclusion, it was clear that the administration of allura red to rats caused many disturbances in the physiological and biochemical parameters. Finally, more extensive assessments of azo dyes additives in general and allura red in particular is warranted.*

**KEYWORDS:** Food Additives, Allura Red, Hematological and Biochemical Parameters, Albino Rat & Serum

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### INTRODUCTION

A number of food coloring additives are added to improve the appearance of the food items and drinks. Colorant provides an aesthetic appearance to food stuff. Allura Red AC is a commonly used color all over the world, mainly for red. It is used to color gelatins, puddings, dairy products, confections, beverages, condiments and numerous other commodities. Exposures to various food colors can cause some disorders.

Allura red food dye is often used a coloring dye in foods like sweets and drink. But it is, like any other synthetic food color, known to cause a set of side effects and health hazards. The main side effects of the allura dye are hyperactivity symptoms in kids, aggravating of asthma and breathing problems, allergy for aspirin related intolerance and bleak risks of causing bladder cancer in animals. Amaranth is also a kind of red food coloring which is quite popular among the food dyes, but mostly allura red is used.

Many researchers (Reyes *et al.*, 1996; Tanaka, 2005; Zraly, 2006 and Gao *et al.*, 2011) studied the metabolic and toxicological disorders induced by the administration of specific food colourant additives to rats and other mammals. Many azo compounds are genotoxic in short-term tests and carcinogenic in laboratory animals (Combes & Haveland - Smith, 1982; Sasaki *et al.*, 2002 and Saxena & Sharma, 2015).

The use of allura red, which is classified as nongenotoxic (**Combes and Haveland-Smith, 1982**), is permitted in the U.S. The U.S. National Toxicology Program found that allura red is negative to Salmonella (**NTP, 2000**). Although an impurity in allura red can be reduced to yield an ether-extractable mutagen (**Prival *et al.*, 1988**), the dye is not carcinogenic in rats (**Borzelleca *et al.*, 1989**) or mice (**Borzelleca *et al.*, 1991**). One teratology study with allura red in rats showed negative results (**Collins *et al.*, 1989a**), while another showed reduced ossification of the hyoid (**Collins *et al.*, 1989b**). Allura red produces evidence of both physical and behavioral toxicity in developing rats (**Vorhees *et al.*, 1983**). In 2001, **Tsuda *et al.*** reported that some azo dyes as allura red induce colon DNA damage at a very low dose in mice. Yet further investigations are required from other points of view in order to confidently predict the potential danger of these food dyes to mankind.

The present study was aimed to determine the effect of allura red on some physiological and biochemical parameters in male albino rats. Also, the study is an attempt to determine if high doses of allura red can affect in liver and kidney functions.

## Experimental

### Materials

#### Synthetic Dye Used 6-Hydroxy-5-[(2-Methoxy-5-Methyl-4-Sulfophenyl) Azo]-2-Naphthalenesulfonate

Allura Red AC Sigma- Aldrich (Germany);

Synthetic azo dye.

Red azo dye.

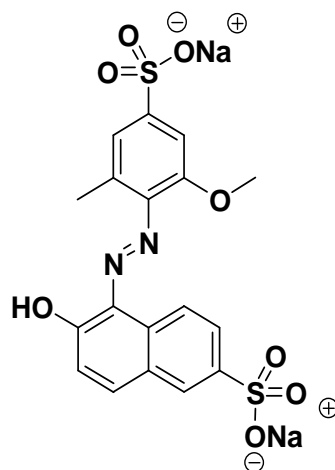
Soluble in water.

**Alternate Names:** Allura Red AC, FD&C Red.

**E Number:** E129

**Uses:** Coloring

Found In: soft drinks, candy, children's medications, cereal, beverages, snacks, gelatin desserts, baked goods, ice cream



**Figure 1: Allura Red AC**

## **Animals**

Forty mature male albino rats (*Rattus norvegicus*) ranging in weight from 100-110 g was tested in this investigation. The rats were kept under normal laboratory conditions, fed on standard diet and water ad libitum. All rats were starved for 12 hrs. Before treatment, but allowed free excess to water. They were allocated at random into four groups: The first and third groups (20 rats) were considered as controls. The second group (10 rats) was orally administered a dose of 50 mg/kg body weight/day of allura red for 10 days. The fourth group (10 rats) was also orally administered a dose of 50 mg/kg body weight/day of allura red for 40 days. After each period of treatment each group was sacrificed to assay the impact of allura red.

## **Haematological Studies**

A part of blood samples were collected on heparinized capillary tubes for haematocrit value, which was determined according to the method of Rodak (1995). Another part of blood was collected on EDTA for the haematological experimentation. Red and white blood cells counts were performed using improved haemocytometer according to Dacie and Lewis (1991). Haemoglobin concentration was estimated according to Dacie and Lewis (1991). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to Dacie and Lewis (1993).

## **Biochemical Analysis**

Samples of blood were withdrawn and left to clot in a clear dry centrifuge tubes for each rat, then centrifuged at 3500 r.p.m. for 15 minutes. A portion of the clear supernatant serum was used immediately for glucose determination according to the enzymatic colorimetric method described by Trinder (1969). The remaining serum was frozen at -20°C for subsequent analysis.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated according to the method described by BergMeyer and Bernt (1974). Serum alkaline phosphatase (ALP) was determined by the method of Belfield and Golbderg (1971).

Serum content of urea and creatinine were estimated according to the methods described by Patton & Crouch (1977) and Bartels & Bohmer (1972), respectively.

Serum total protein and albumin levels were estimated according to the methods described by Doumas (1975) and Doumas et al. (1971), respectively. Serum globulin was calculated according to Latner (1975).

## **Data Analysis**

The obtained results were statistically analysed by using the student "t"-test according to the method of Snedecor and Cochran (1980).

## **RESULTS**

Results presented in table (1) show the alterations of the various hematological parameters in the blood of the studied rats induced by allura red. Rats supplemented with allura red for 10 days exhibited highly significant decrease ( $p < 0.01$ ) in red cell count (R.B.Cs) and also in the mean corpuscular haemoglobin concentration (MCHC). Furthermore, the same dose recorded a significant decrease ( $p < 0.05$ ) in haemoglobin (Hb) content, while it showed a highly significant

( $p < 0.01$ ) increase in the mean of corpuscular volume (MCV). Same results were recorded in the other group for 40 days. Whereas, leucocytes (W.B.Cs) counts and the mean corpuscular haemoglobin (MCH) of albino rats were not affected by allura red throughout the experiment.

The data represented in table (2) displayed the effect of treatment with allura red on enzymatic activities which reflect the liver function of male albino rats. A highly significant increase ( $p < 0.01$ ) in AST; ALT and ALP was detected in groups treated with allura red for the two periods.

The obtained data in table (3) as a short and long-term administration of allura red showed a highly significant ( $P < 0.01$ ) rise of rat serum urea; creatinine and glucose levels.

**Table (4)** demonstrated the effect of allura red on total serum proteins, albumin, globulin levels and A/g ratio. The first dose of allura red caused a significant increase ( $p < 0.05$ ) in total serum proteins and serum globulin after treatment for 10 days. No significant changes were recorded in the other groups and parameter.

**Table 1: Blood Picture Value of Male Rats Albino; Rats Treated with Allura Red**

Group	R.B.Cs ( $\times 10^6/\text{mm}^3$ )	Hb (g/dl)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (%)	W.B.Cs ( $\times 10^3/\text{mm}^3$ )
<b>After 10 days</b>							
1	$8.3 \pm 0.04$	$14.41 \pm 0.31$	$38.40 \pm 1.32$	$46.27 \pm 0.18$	$17.36 \pm 0.18$	$37.53 \pm 0.83$	$4.97 \pm 0.98$
2	$7.4 \pm^{**}0.12$	$13.05 \pm^{*}0.45$	$41.90 \pm^{*}1.04$	$56.62 \pm^{**}0.59$	$17.64 \pm^{NS}0.29$	$31.15 \pm^{**}0.76$	$5.81 \pm^{NS}1.71$
<b>After 40 days</b>							
3	$8.5 \pm 0.09$	$14.35 \pm 0.42$	$37.60 \pm 1.61$	$44.23 \pm 0.86$	$16.88 \pm 0.26$	$38.16 \pm 1.04$	$16.88 \pm 0.26$
4	$8.0 \pm^{*}0.1$	$13.29 \pm^{*}0.17$	$41.2 \pm^{NS}1.22$	$51.5 \pm^{**}0.67$	$16.61 \pm^{NS}0.14$	$32.26 \pm^{**}0.71$	$5.13 \pm^{NS}1.60$

- All values were expressed as mean  $\pm$  standard error of 10 rats.

- Group (1&3) : control rats.

- Group (2) : rats treated with allura red for 10 days.

- Group (4) : rats treated with allura red for 40 days.

<sup>NS</sup> = Insignificant ( $P > 0.05$ )

<sup>\*</sup> Significant ( $P < 0.05$ )

<sup>\*\*</sup> Highly Significant ( $P < 0.01$ )

**Table 2: Effect of Treatment with Allura Red on AST, ALT and ALP Activities of Male Albino Rats at Two Periods**

Groups	AST (U/ml)	ALT (U/ml)	ALP (U/ml)
<b>After 10 days</b>			
(1)	$152.86 \pm 0.15$	$84.56 \pm 0.34$	$176.96 \pm 0.57$
(2)	$184.92 \pm 0.38^{**}$	$136.01 \pm 0.87^{**}$	$184.38 \pm 0.24^{**}$
<b>After 40 days</b>			
(3)	$156.82 \pm 0.32$	$88.51 \pm 0.21$	$179.06 \pm 0.57$
(4)	$188.41 \pm 0.39^{**}$	$141.36 \pm 0.56^{**}$	$185.92 \pm 0.45^{**}$

All values were expressed as mean  $\pm$  standard error of 10 rats.

**Group (1&3):** Control rats.

**Group (2):** Rats treated with allura red for 10 days.

**Group (4):** Rats treated with allura red for 40 days.

\*\* Highly Significant (P < 0.01)

**Table 3: Effect of Treatment with Allura Red on Blood Urea, Serum Creatinine and Serum Glucose of Male Albino Rats at Two Periods**

Groups	Serum Urea (Mg/L)	Serum Creatinine (Mg/L)	Serum Glucose (Mg/100ml)
<b>After 10 days</b>			
(1)	38.91 ± 1.34	16.00 ± 1.42	84.60 ± 1.38
(2)	46.32 ± 2.96*	22.78 ± 0.38**	115.78 ± 4.05**
<b>After 40 days</b>			
(3)	38.51 ± 2.40	15.21 ± 1.23	87.08 ± 2.62
(4)	51.98 ± 2.76**	27.11 ± 0.78**	113.05 ± 1.36**

All values were expressed as mean ± standard error of 10 rats.

**Group (1&3):** Control rats.

**Group (2):** Rats treated with allura red for 10 days.

**Group (4):** Rats treated with allura red for 40 days.

\* Significant (P < 0.05)

\*\* Highly Significant (P < 0.01)

**Table 4: Effect of Treatment with Allura Red on Serum Total Proteins, Albumin, Globulin and A/G Ratio of Male Albino Rats**

Groups	Serum Total Protein (g/dL)	Serum Albumin (g/dL)	Serum Globulin (g/dL)	A/g Ratio
<b>After 10 days</b>				
1	7.15 ± 0.15	3.77 ± 0.15	3.37 ± 0.12	1.15 ± 0.31
2	8.25 ± 0.24	3.89 ± <sup>NS</sup> 0.14	4.38 ± *0.37	0.81 ± <sup>NS</sup> 0.93
<b>After 40 days</b>				
3	7.22 ± 0.10	3.73 ± 0.09	3.47 ± 0.32	1.02 ± 0.42
4	8.36 ± <sup>NS</sup> 0.21	3.91 ± <sup>NS</sup> 0.13	4.44 ± <sup>NS</sup> 0.49	0.91 ± <sup>NS</sup> 0.53

All values were expressed as mean ± standard error of 10 rats.

**Group (1&3):** Control rats.

**Group (2):** Rats treated with allura red for 10 days.

**Group (4):** Rats treated with allura red for 40 days.

<sup>NS</sup> = Insignificant (P > 0.05)

\* Significant (P < 0.05)

## DISCUSSIONS

The present study is concerned with the effect of allura red (azo dye) on some physiological and biochemical parameters on male albino rats. Azo dyes have been tested by the oral route in mice, rats and dogs and by the subcutaneous route in rats. Two Oral administrations of Azo dye studies in rats indicated a carcinogenic effect (IARC, 1975).

Hematological parameters were valuable tools for assessing injuries that caused by certain substances. The RBC counts were the most useful as raw data for calculation of the erythrocyte indices MCV and MCH. Decreased RBC is usually seen in anemia of any cause.

In the present investigation marked variations in the hematological parameters were observed under allura red administration. The data showed a marked decrease in erythrocytes (RBCs) count, hemoglobin (Hb) and MCHC in both two groups of treated rats after 10 and 40 days. These changes induced by allura red may be due to the prevention of red blood cell synthesis via inhibition of erythropoiesis in the bone marrow, in agreement with Chakravarty et al. (2005) who showed a decrease in hemoglobin content and total erythrocyte count at all dose levels of the food dye used.

On the contrary to the above findings, Ford et al., 1987 stated that carmosine (given in high doses for 6 months) did not cause any changes in the hematological investigations of rats. The marked discrepancies observed between the various research studies might be attributed to dose variations as well as the duration of food colorant intake. The total leucocytic count and MCH in the present study remained unchanged in all experimental groups. This finding was in agreement with Borzelleca and Hallagan (1988). Also, Himri et al. (2011) showed that tartrazine (azo dye) at doses of 5.0, 7.5 and 10 mg/kg bw administered in rats for 90 days did not affect the total leucocytes count.

Serum aminotransferases activities are known as toxicity markers in the study of hepatotoxicity caused by chemicals (Govindwar and Dalvi, 1990). An increase in the activities of these enzymes was termed as the early recognition of toxic hepatitis.

Results of the present investigation revealed a marked elevation in AST: ALT and ALP activities throughout the entire experimental period in groups treated with allura red. The elevation of the aminotransferases activities in blood has been considered as an indicator of tissue damage. Similar results were reported by Abdel-Rahim et al. (1989) who found significant increase in both serum AST and ALT of rats fed on brown food dye for three months, he attributed these changes in liver function to hepatocellular impairment which subsequently caused the release of greater than normal levels of intracellular enzymes into the blood. The elevation in serum ALP may be an evidence of obstructive damage in the liver tissue due to allura red administration. This observation is in agreement with that reported by Chakravarty et al. (2005). These results are in concordance with the data of Amin et al. (2010) which revealed that rats that consumed high dose of tartrazine (500 mg/kg bw) for 30 days exhibited a significant increase in serum ALT, AST and alkaline phosphatase activities. The present findings are in accordance with those of Mekkawy et al. (1998) who indicated that two doses of synthetic dyes (low or high doses); tartrazine and carmoisine (Ponceau, Carmoisine, Erythrosine, sunset yellow, tartrazine, fast green, indigo, brilliant blue and brilliant black) showed a significant increase in serum AST, ALT, and alkaline phosphatase activities. Also, these results are in accordance with those of Aboel-Zahab et al. (1997) who found that liver enzymes ALT, AST, and Alkaline phosphatase were elevated in rats whose diets were supplemented with Chocolate colors A and B (Sunset Yellow, Tartrazine, Carmoisine and Brilliant Blue in varying concentrations).

The present investigation showed a significant increase serum urea content of treated rats with allura red for 10 days accompanied with a highly significant increase after 40 days. Protein catabolism is the major source of ammonia for urea synthesis (Kaneko et al., 1997). The elevation of urea in this study could be attributed to an increase of nitrogen retention and/or due to corrupted renal function as explained by Gilman et al. (1991). A significant increase in serum urea level was observed in severe defect of glomerular filtration (Kaneko et al., 1997). Creatinine is a waste product of creatin metabolism whose measurement provides an exceptionally useful index of kidney function (Hood, 1980). In the present study, concentration of creatinine showed a highly significant increase in allura red treated group till the end of the experiment. Furthermore, the present findings are in accordance with data reported by Ashour and Abdelaziz (2009) who observed a significant elevation in serum creatinine and urea level of rats dosed with organic azo dye (Fast Green) orally

for 35 days. Also, Amin et al. (2010) showed a significant elevation in serum creatinine and urea level of rats that ingested either low or high doses of tartrazine. An increase level of urea or creatinine in the plasma indicates renal dysfunction (Timbrell, 2009).

Glucose is a key molecule in carbohydrate metabolism. It is formed both as a result of the digestion of complex carbohydrates or as a results of synthesis within the body (gluconeogenesis) (Hood, 1980). The present results showed that rats administered with allura red revealed a high significant elevation (hyperglycemia) till the end of the experiment. The elevation of glucose level can be explained by stimulation of glycogenolysis and gluconeogenesis by the liver with a temporarily loss of endocrine functions of pancreas leading to hyperglycemia. This hyperglycemia might be explained by changes in blood sugar concentration caused by foreign compounds. These results agree with Amin et al. (2010). He showed a significant increase in glucose concentration when administrated tartrazine at low (15 mg/kg bw) and high (500 mg/kg bw) dose orally in males rats for 30 days.

Serum total protein in this study exhibited a significant increase in rats received allura red for 10 days only , these results are in accordance with those of Amin et al. (2010) who found a significant increase in serum total protein when administrated tartrazine at low and high dose in males rats for 30 days . Also, our finding is in agreement with the findings of El-Saadany (1991). The accumulation of serum protein can be attributed to the stimulation of protein biosynthesis to produce the specific enzymes required for all processes. The globulin fraction in the serum of rats studied, recorded an increase induced by allura red. The specific elevation in globulin fraction pointed towards an increase in immunoglobulin synthesis, the defense mechanism which aims to protect the body from the toxic effects of this synthetic food colorant.

## CONCLUSIONS

In conclusion, it was clear that the administration of allura red to rats caused many disturbances in the physiological and biochemical parameters. Finally, more extensive assessments of azo dyes additives in general and allura red in particular is warranted.

## Conflicts of Interest

The authors declare no conflict of interest.

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